Sensitive measurement of quantity dynamics of FLT3 internal tandem duplication at early time points provides prognostic information

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Background: The level of minimal residual disease (MRD) in acute myeloid leukemia (AML) at early time points (TPs) may be an important prognostic factor. Although internal tandem duplication of FLT3 (FLT3-ITD) as an MRD marker has been questioned for its instability based on semi-quantitative methods, we hypothesized that FLT3-ITD dynamics measured by sensitive quantitative real-time PCR at early TPs before appearance of instability may provide prognostic information.

Patients and methods: We measured mutant quantity in 493 serial samples from 55 patients with a median follow-up time of 64.8 months. The FLT3-ITD quantities after induction (TP1) and after the first post-induction chemotherapy (TP2) were analyzed.

Results: We found that lower FLT3-ITD levels at TP2 predicted longer overall survival (OS) and disease-free survival (DFS) regardless of cytogenetic risk. Multivariate analysis showed that ≥3 log reduction of FLT3-ITD at TP2 independently predicted better DFS and a trend toward better OS. FLT3-ITD disappeared at relapse in 16.7% of patients and none in those harboring mutant NPM1 compared with 29.4% in those with wild-type NPM1 (P = 0.032).

Conclusions: Though the mutation may disappear at relapse in a few patients, FLT3-ITD levels at early TPs after chemotherapy provide prognostic information. FLT3-ITD is significantly more stable in those with mutant NPM1.

Key words: acute myeloid leukemia, FLT3-ITD, minimal residual disease, NPM1, real-time PCR

Introduction

A multitude of markers are now available for stratifying patients diagnosed with acute myeloid leukemia (AML), a highly heterogeneous disease with respect to clinical manifestations, molecular pathogenesis, and treatment outcomes. Conventionally, karyotypic risk is the strongest predictor of disease prognosis. Recent identification of mutations in CEBPA [1], WT1 [2, 3], FLT3 [1, 4], and NPM1[1, 5–11] provides yet another molecular platform for classifying this heterogeneous disease. Internal tandem duplications in the FMS-like tyrosine kinase (TK) 3 (FLT3-ITD) and mutations of nucleophosmin (NPM1) genes are particularly relevant for disease classification due to their high incidences in adult AML [1, 5]. Generally, FLT3-ITD confers a worse prognosis, while NPM1 mutations usually lead to a better outcome [4, 5]. Analyses of both genes in combination have confirmed a favorable prognosis in patients who retain wild-type FLT3 but develop NPM1 mutations [1, 4].

Recently, the International Working Group included molecular remission as one of the criteria for measuring treatment response in AML [12]. The level of minimal residual disease (MRD) at early time points (TPs) such as after consolidation chemotherapy seems to be an important prognostic marker in AML [13, 14], although large prospective trials demonstrating its clinical utility are still pending except for acute promyelocytic leukemia (APL) [15]. Some of these studies employed multicolor flow cytometry (MCF) as a tool to assess MRD with the assumption that this technique could be applied to most of the AML patients. However, interpretation of MCF for MRD detection is technically demanding and highly individualized. Quantitative real-time PCR (qPCR) of molecular targets, such as the fusion genes PML-RARα and AML1-ETO, is a well-established method for assessment of MRD [16–19]. We and others have established NPM1 as another marker for MRD assessment [14, 20, 21] and the levels of MRD during treatment can provide prognostic information [14, 21]. However, FLT3-ITD as an MRD marker has been...
questioned for its instability based on semi-quantitative methods with limited sensitivity [22–25]. Nonetheless, the instability likely occurs at late stage of disease at relapse. Therefore, we hypothesized that dynamics of \( \text{FLT3-ITD} \) measured by sensitive qPCR at early TP, well before the appearance of instability, can provide prognostic information. In this study, we found that the MRD levels of \( \text{FLT3-ITD} \) at early TPs, like those of mutant \( \text{NPM1} \), could independently reflect patients’ prognoses. Moreover, the \( \text{FLT3-ITD} \) mutation was significantly more stable at disease relapse when an \( \text{NPM1} \) mutation was also present. Although the patient number in our study is relatively small, our findings suggest a new and broadly applicable MRD marker for prognostic stratification. The significant impact of \( \text{NPM1} \) mutation status on the stability of \( \text{FLT3-ITD} \) may provide insight into the pathogenetic role of the interaction between \( \text{FLT3-ITD} \) and \( \text{NPM1} \) mutant in leukemogenesis.

patients and methods

patients

Adult AML patients (≥17 years of age) were screened for \( \text{FLT3-ITD} \) and mutant \( \text{NPM1} \) as previously described [11]. In our institute, the standard treatment of non-APL AML includes conventional induction chemotherapy (idarubicin 12 mg/m\(^2\) or doxorubicin 30 mg/m\(^2\)/day on days 1–3 and cytarabine 100 mg/m\(^2\)/day on days 1–7). Once complete remission (CR) was achieved, two to four courses of consolidation chemotherapy with high-dose cytarabine (2000 mg/m\(^2\) every 12 h, days 1–4 for a total of eight doses), with or without an anthracycline, are administered. For APL patients, concurrent all-trans-retinoic acid and chemotherapy (idarubicin 12 mg/m\(^2\)/day on days 1–2) were given as induction therapy. Consolidation chemotherapy with idarubicin-based regimen was given for two to four courses after remission was achieved. This study has been approved by the Institutional Review Board of the National Taiwan University Hospital.

determination of \( \text{FLT3-ITD} \) and mutant \( \text{NPM1} \) sequences

The PCR products from genomic DNA were cloned into TA vectors (Yeastern Biotech, Taipei, Taiwan). The presence of mutant \( \text{NPM1} \) was determined by direct sequencing of the mutant clones [11]. For \( \text{FLT3-ITD} \), mutant clones were first differentiated from wild-type clones with the following pair of internal primers: \( 5'-\text{CAAATTTAGGTATGAAAGCCAGCTA-3'} \) and \( 5'-\text{CAAACTCTAAATTTTCTCT-3'} \). The mutant clones that yielded longer products were sequenced to determine the mutation sequences.

semi-quantification of mutant \( \text{NPM1} \) and \( \text{FLT3-ITD} \) mutants by GeneScan

\( \text{NPM1} \) semi-quantification by GeneScan was carried out with 50 ng of genomic DNA and 750 nM of each \( \text{NPM1} \) primer pair (forward: VIC-labeled \( 5'-\text{AGGACAGCAGATATACAACTGTTAC-3'} \) and reverse: \( 5'-\text{AGTTAATCTCTCTGTTGTAAGATGAAA-3'} \)) in an 8 μl reaction. Identical reaction conditions were used for \( \text{FLT3-ITD} \) semi-quantification with an \( \text{FLT3-ITD} \) primer pair (forward: NED-labeled \( 5'-\text{AAAAACTCTTAAAATTTCCT-3'} \) and reverse: \( 5'-\text{CAATTTAGGTATGAAAGCC-3'} \) (Applied Biosystems, Foster City, CA). At 30 cycles of PCR, the products were denatured by formamide and analyzed as previously described [26].

qPCR of mutant \( \text{NPM1} \) and \( \text{FLT3-ITD} \) mutations

Quantification of mutant \( \text{NPM1} \) was carried out as previously described [14], with the exception of patients recruited within the last 2 years, where the large ribosomal protein (\( \text{RPLP0, NM_001002} \)) (Applied Biosystems) was used as an internal control (INT). For \( \text{FLT3-ITD} \) mutant quantification, we designed a common probe (\( 5'-\text{TGGACGATTTCCTTTTC-3'} \)) labeled with carboxyfluorescein (FAM)/minor groove binding protein (Applied Biosystems) and a common reverse primer (\( 5'-\text{GGTGAGTACGTGCATT-3'} \)). The forward primers were patient specific and designed based on the mutation sequences. All the primers encompassed the junction of insertion/duplication in order to differentiate mutant from wild-type sequences (Figure 2A). The INT was assayed with a forward primer located in intron 15 (\( 5'-\text{GAGATGGAATGTGCAAAATTGTTT-3'} \)) and the above probe and reverse primer. This design allowed for the amplification of both wild-type and mutant \( \text{FLT3} \) alleles. Each 20 μl reaction contained 100 ng of genomic DNA, 500 nM each of forward and reverse primers, 10 μl of master mix (Applied Biosystems), and 250 nM of probe. The reactions were carried out on an iQ5 machine (Bio-Rad, Hercules, CA) using the following PCR conditions: step 1, 10 min at 95°C for 1 cycle and step 2, 10 sec at 95°C and 1 min at 60°C for 40 cycles. Standardization of MRD was achieved by absolute quantification. First, we got every clone containing specific \( \text{FLT3-ITD} \) mutation sequences and used serial dilution of every clone (from \( 10^9 \), \( 10^8 \), \( 10^7 \), \( 10^6 \) to 10 copies) for creation of standard curves, which would then used as a reference for absolute quantification of the mutant copy numbers of the patient samples. In the meantime, a standard curve for internal control (which serves as a loading control) was included. The loading DNA amount could be calculated accordingly. Thus, the mutant copy numbers in a constant amount of internal control can be calculated and compared among different samples.

statistical analysis

Correlation between continuous variables was determined by the Spearman correlation coefficient. The \( \chi^2 \) test was used to calculate the significance of association among discrete parameters. Nonparametric Mann–Whitney \( U \) test was carried out to compare two groups of continuous variables. The Fisher’s \( F \) test was used to compare variances of two series of samples. The difference of the means of two samples was measured by paired \( t \)-test.

To rigorously test the prognostic significance of MRD levels of \( \text{FLT3-ITD} \), both univariate and multivariate analyses for survival were restricted in 44 patients with intermediate-risk karyotypes, which were defined according to the Medical Research Council [27]. Multivariate analysis was calculated with the Cox proportional hazards model. To exclude the confounding effects of hematopoietic stem cell transplantation (HSCT) on the survival analysis, those patients who had received this procedure were censored at the time of HSCT, as described previously [28, 29]. The Kaplan–Meier curve was plotted using Statistical Package for the Social Sciences software (Chicago, IL).

results

patterns of mutation sequences of \( \text{FLT3-ITD} \)

From 1996 to 2008, 569 consecutive adult AML patients were analyzed for gene mutations. Among them, 126 (22.1%) harbored \( \text{FLT3-ITD} \)s and 42.9% (54 of 126) of these patients also bore mutant \( \text{NPM1} \) simultaneously. We determined 118 \( \text{FLT3-ITD} \) sequences from 104 patients who had leukemic cell DNA available for analysis. In these 104 patients, 53 were males and 51 were females, with a median age of 48.5 years (range 17–90). The median values of white blood cell (WBC), platelet, hemoglobin, and lactate dehydrogenase (LDH) were 4593/μl (range 470–35 230), 52 000/μl (range 3000–331 000), 8.1 g/dl (range 4.3–12), and 1308 U/l (range 316–6140).
respectively. In terms of French-American-British subtype, 2 patients had AML M0, 21 had M1, 30 had M2, 7 had M3, 36 had M4, and 8 had M5. Seventy patients had a normal karyotype and 47 harbored NPM1 mutation. All the insertions/duplications occurred in exon 14 with the sizes ranging from 12 to 210 nucleotides (median 48). All mutations occurred in-frame and therefore a functional FLT3 gene product was predicted to be generated from every mutation. Most of the mutations were direct repeats (74 of 118, 62.7%) without insertion. One patient had direct triple repeats. The most frequent amino acid involved was arginine at position 595 and occurred in ~75.4% of the mutant sequences (Figure 1). The positions of the insertions/duplications occurred at the juxtamembrane (JM) region (position 571–609) in 84 patients (71.2%) and in the β-sheet of the TK domain (position 610–615) in 32 patients (27.1%). One patient had an insertion/duplication at the nucleotide-binding loop (nucleotide-binding loop; position 616–623) (Figure 1). Another patient had an insertion/duplication at position 640 (Figure 1).

**dynamics of FLT3-ITD measured by real-time PCR**

To explore the clinical implication of MRD levels of FLT3-ITD, we then focused on 55 patients who were treated by standard intensive chemotherapy and had serial marrow samples available for analysis. There were 32 males and 23 females included in this portion of the study with a median age of 40 years (range 17–85). The median follow-up time was 64.8 months. The first two post-treatment marrow examinations were carried out at a median of 1.2 months (0.7–3.6) after diagnosis and 1.35 months (0.6–4.0) after the first post-induction therapy. We used patient-specific forward primers combined with a common probe and a common reverse primer for sensitive determination of FLT3-ITD mutation dynamics during the clinical follow-up. The same probe and reverse primer were combined with a forward primer located in intron 14 to quantify total FLT3 copy number (both mutant and wild type) present in every reaction (Figure 2). To ensure that the sensitivity of all 55 combinations of patient-specific primers, common probe, and common reverse primer was uniform, a respective mutant clone was serially diluted from 10⁶ copies to 50 or 100 copies in 100 ng of human genomic DNA that lacked FLT3-ITD. The slopes of amplification ranged from −3.2593 to −3.9587 (median of −3.632) with a r² >0.99 in each test. We quantified FLT3-ITD mutant alleles by qPCR in 489 serial samples taken from the 55 patients. The FLT3-ITD copy number decreased significantly after induction chemotherapy (P < 0.0001) but rose at relapse (P = 0.004) (data not shown). Generally, when the marrow blasts were evident, FLT3-ITD was also detected. Conversely, when the number of marrow blasts was <5% and the disease was considered in CR

**Figure 1.** The region of duplication in FLT3-ITD. The x-axis denotes amino acid residues from 573 to 620, with the underlying numbers representing the ordinates of FLT3 protein sequence. The y-axis represents the proportions of involved amino acids (a.a.) in the duplicated region. Most mutants (84 of 118, 71.2%) initiate insertions/duplications at amino acids within the juxtamembrane (JM) region (a.a 571–609). There are 27.1%, 0.85%, 0%, and 0.85% starting the insertion/duplication within β-sheet (a.a. 610–615), nucleotide-binding loop (NBL; a.a. 616–623), β2-sheet of tyrosine kinase region (a.a. 616–623), and a.a. 640, respectively.
morphologically, there were still considerably high numbers of FLT3-ITD in many samples (data not shown). Figure 3 shows the FLT3-ITD dynamics in six representative patients. Generally, the gene dynamics correlated well with the clinical treatment response. However, qPCR was more sensitive than morphological observation. As shown in Figure 3, the patients exhibited an increase in FLT3-ITD even before clinical relapse was evident (Figure 3A–D). In Figure 3E, there are no detectable signals after the induction treatment and the patient experienced continuous CR. However, we noted that some samples obtained at clinical relapse lost FLT3-ITD; one representative example is shown in Figure 3F. This patient experienced two episodes of clinical relapse with the first relapse accompanied by a rise in FLT3-ITD copy number. Interestingly, at the reemergence of disease progression, the signals became undetectable, implying

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**Figure 2.** Quantification of FLT3-ITD alleles by quantitative real-time PCR. The location and design of patient-specific forward primers, common probe, and common reverse primer. A forward primer for internal control (INT) localizes in intron 14.

**Figure 3.** The levels of FLT3-ITD in six representative patients during clinical follow-up. In panels (A–D), the patients’ FLT3-ITD copy numbers generally correlate with clinical responses. Many samples of morphological complete remission (CR) still contain considerable amount of mutant copies. In panel (E), patient Unique Patient Number (UPN) 119 experiences continuous CR and there are no detectable signals after the induction treatment. In panel (F), the patient experienced two episodes of clinical relapse. The first relapse was accompanied with a rise in FLT3-ITD copy number. However, as the disease became refractory, the signals were undetectable, implying a loss of the original FLT3-ITD during disease evolution. The FLT3-ITD amount at initial diagnosis is designated 0. The quantities of mutants in subsequent samples are denoted in log scale based on the initial amount. The arrow denotes chemotherapy; R, relapse; alo, allogeneic hematopoietic stem cell transplantation; DLI, donor lymphocyte infusion. The numbers on the x-axis represent months after initial diagnosis. Please note that the intervals are not drawn in scale. Arrow indicates chemotherapy.
that FLT3-ITD was unstable in this patient and lost as the disease evolved (Figure 3F).

**dynamics of FLT3-ITD are predictive of disease prognosis**

To rigorously test the prognosis prediction power of FLT3-ITD levels as an MRD marker, we restricted our survival analysis to 44 patients who had an intermediate-risk karyotype, according to MRC criteria [27]. Seventeen out of these 44 patients received allogeneic HSCT (allo-HSCT). To exclude the confounding effects of HSCT on survival analysis, these patients were censored at the time of transplantation procedures. Moreover, allo-HSCT was carried out after TP1 and TP2, two TPs when MRD levels were assessed for survival analysis in this study. Therefore, the TPs defined in survival analysis (TP1 and TP2) were independent of the use of allo-HSCT therapy. Detailed analysis of the dynamics of FLT3-ITD suggested that both overall survival (OS) and disease-free survival (DFS) were significantly longer in patients who obtained a >3-log reduction of FLT3-ITD at TP2 (not reached versus 14.7 months, \( P = 0.016 \) and 7.5 versus 3.0 months, \( P < 0.001 \), respectively) (Figure 4A and B). In contrast, only DFS, but not OS, was significantly influenced by mutation reduction at TP1 (data not shown). The OS and DFS were longer in patients who had once obtained a 4-log reduction of mutant signals during clinical treatment (32.0 versus 12.5 months, \( P = 0.012 \), and 9.0 versus 3.5 months, \( P = 0.005 \), respectively) (Figure 4C and D). The prediction power of FLT3-ITD as a marker of MRD was further tested by multivariate analysis. Reduction of FLT3-ITD by ≥3 logs at TP2 was a strong and good prognostic factor for DFS [hazard ratio 0.264, 95% confidence interval (CI) 0.114–0.614, \( P = 0.002 \)], probability of relapse (hazard ratio 0.726, 95% CI 0.554–0.952, \( P = 0.021 \)), and showed a trend for better OS (hazard ratio 0.308, 95% CI 0.092–1.035, \( P = 0.057 \)) independent of a patient’s karyotype, age, initial WBC count, LDH level, and NPM1 mutation status (Table 1).

**Figure 4.** The prognostic significance of the dynamics of FLT3-ITD. Overall survival (OS) (A) and disease-free survival (DFS) (B) are significantly longer in patients who can obtain a >3-log reduction in mutation copy number after the first post-induction (post-IND) (not reached versus 14.7 months, \( P = 0.016 \), and 7.5 versus 3.0 months, \( P < 0.001 \), respectively). OS (C) and DFS (D) are also significantly longer for those who can reach a >4-log reduction in mutant copy number during the clinical treatment (32.0 versus 12.5 months, \( P = 0.012 \), and 9.0 versus 3.5 months, \( P = 0.005 \), respectively).
Table 1. Impact of prognostic factors on OS and DFS by multivariate analysis for the patients with intermediate-risk cytogenetics

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CI, confidence interval; DFS, disease-free survival; LDH, lactate dehydrogenase; OS, overall survival; NS, non-significant; TP2, time point 2, after the post-induction chemotherapy; WBC, white blood cell.

Figure 5. Direct comparison of FLT3-ITD and mutant NPM1 quantities.
(A) The dynamics of FLT3-ITD and mutant NPM1 in a representative patient (UPN 805) had good correlation of copy number changes during the clinical treatment. (B) An $xy$ distribution graph for mutant NPM1 and FLT3-ITD copy number in 116 samples from 20 patients with both mutations. Some samples with high mutant NPM1 copy numbers had no detectable FLT3-ITD signal, suggesting a loss of the original FLT3-ITD mutations. CR, morphological complete remission; R, clinical relapse.

Table: Impact of prognostic factors on OS and DFS by multivariate analysis for the patients with intermediate-risk cytogenetics

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Discussion

Our large-scale mutation analysis of FLT3-ITD has shown that the insertions/duplications were in-frame, clustered within exon 14, centered around 395R, and generally similar to samples taken from patients of Western populations [30, 31]. Most of the insertions/duplications of our patients occurred in

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the JM region, although a significant number of them (27.1%) occurred in the β1-sheet of the TK domain as shown in a recent report [32].

We and others have demonstrated that the presence of mutant NPM1 can be very useful in MRD monitoring and prognostic stratification [14, 21]. Since MRD level has emerged as an important prognostic factor, we were interested in searching for more markers for this purpose. Although FLT3-ITD as an MRD marker has been questioned for its instability based on semi-quantitative methods with limited sensitivity [21–25], the instability likely occurs in late stage at disease relapse. Therefore, we hypothesized that the prognostic implication of FLT3-ITD dynamics measured by sensitive qPCR at early TPs might not be affected by this feature of mutation. In this study, we found that MRD of FLT3-ITD, one common mutation in AML, early after chemotherapy could also serve as a prognostic factor. The significance of FLT3-ITD as a marker of MRD to predict clinical outcome was confirmed by rigorous multivariate analysis and was proved to be independent of karyotypic risk, WBC count, age, LDH level, and NPM1 mutation status. Our study suggests that nearly half of adult AML patients have an MRD marker available for prognosis assessment after induction and the first post-induction treatment when analyses of mutant NPM1 and FLT3-ITD are combined. This percentage increases when we focus on those with a normal karyotype since both mutations tend to occur in this subgroup of AML patients [1, 4, 5]. Kayser et al. [33] reported that FLT3-ITD-positive young adult AML patients with ITD insertion site in the β1-sheet appeared to have unfavorable prognosis. We have tried similar analysis in our patients but did not find any prognostic difference among patients with different sites of insertion, probably related to small number of patients analyzed in our study (data not shown).

While all NPM1 mutants remained in leukemic cells from relapsed patients, approximately one-quarter of FLT3-ITD-positive patients lost the original mutation in our study. These results imply that the NPM1 mutation may be an essential step in leukemia development, while the emergence of FLT3-ITD may act as an extra hit that contributes to the survival of leukemic cells during disease progression. We noted that FLT3-ITDs were present at relapse in all patients with double FLT3-ITD and mutant NPM1 but lost in a significant proportion (29.4%) of patients with FLT3-ITD alone but without an NPM1 mutation. This is in concordance with a recent study in which loss of FLT3-ITD was found only in 8% of patients with a simultaneous mutation in NPM1 [21], an incidence much lower than those previously reported where FLT3-ITD loss was analyzed regardless of NPM1 mutation status [22–25]. It is possible that each mutation contributes individually or in combination to the progression and maintenance of AML. We hypothesize that FLT3-ITD may be essential for disease maintenance in a subset of patients depending on the complex genetic alterations that have taken place in their leukemic cells. More than one FLT3-ITD can exist

Figure 6. Gain of new FLT3-ITD mutations but loss of the original mutations in two patients at relapse. (A) UPN 141 lost the original quantitative real-time PCR (qPCR) signals as the disease escaped remission after chemotherapy (left panel). The original GeneScan graph showed FLT3-ITD with 27 more nucleotides than the wild-type allele at diagnosis (right panel). However, when qPCR did not yield any signal at disease relapse, another FLT3-ITD with an extra 108 nucleotides appeared on GeneScan (right panel). This new FLT3-ITD gradually predominated as the original one disappeared. (B) UPN 140, a patient with both FLT3-ITD and mutant NPM1, retained mutant NPM1 at disease relapse as shown by both qPCR and GeneScan. While the original FLT3-ITD signal disappeared at relapse, the GeneScan graph revealed a new FLT3-ITD peak with 81 nucleotides longer than the wild-type allele. This new FLT3-ITD became more prominent as the original one disappeared after the first treatment of chemotherapy. Please note that the dynamics in gene copy number of FLT3-ITD and mutant NPM1 in this figure were represented with absolute quantification. Arrow indicates chemotherapy; * means wild-type FLT3; ** means FLT3-ITD; . means wild-type NPM1; .. means mutant NPM1.
in some patients at initial diagnosis. The appearance of new FLT3-ITD at disease relapse suggests that these different clones can possess variable drug sensitivity. Those with high resistance will be finally selected at relapse after repeated chemotherapy.

Although our data and those previously reported suggest that mutant NPM1 is more stable than FLT3-ITD [21–25], a recent report demonstrated that up to 9% of AML patients with a mutant NPM1 lost the mutation at subsequent relapse [34]. In fact, loss of original ‘tumor markers’ during disease evolution is not uncommon. Several frequently used markers for MRD monitoring, such as leukemic antigens measured by flow cytometry [35] and immunoglobulin gene and T-cell receptor gene rearrangements [17, 36], are often lost during disease progression. One difficulty in using FLT3-ITD as a marker for MRD monitoring is that prior knowledge of the mutation sequences is required. This requirement also exists for other MRD markers such as MCF, fusion genes, immunoglobulin and T-cell receptor gene rearrangements, and mutations in NPM1 [37]. However, an advantage of FLT3-ITD as an MRD marker is that cross-contamination can be greatly reduced with the use of patient-specific primers [37]. The slopes of our amplification curves are close to −3.322, which means 100% amplification efficiency, suggesting that 2−ΔΔCT can be substituted for absolute quantification without the need to establish a respective standard curve. Patient-specific primers are the only variables used in our methods with the probe and reverse primer common to all patients. In addition, all components of the assays are feasible in general laboratories. Considering these advantages and the prognostic significance of FLT3-ITD allelic changes during the clinical treatment of AML, we believe that FLT3-ITD is an economic, easy, and broadly applicable marker for the prediction of prognosis and MRD monitoring.

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disclosure

The authors declare no conflict of interest.

references


